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Health and Environmental
Effects Profile for Bromophos

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE
FOR BROMOPHOS

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This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste by the Office of Health and Environmental Assessment. The HEEP's are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched."

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include, Acceptable Daily Intakes (ADIs) for chronic exposures. An ADI is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, ADIs are not estimated in this document series. Instead, a carcinogenic potency factor or q_1^* is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEP's will become part of the EPA RCRA docket.

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EXECUTIVE SUMMARY

Bromophos is a broad-spectrum contact insecticide (Worthing and Walker, 1983; Hartley and Kidd, 1983) that is not manufactured in the United States. It is susceptible to microbial degradation in culture media (Stenersen, 1969), but additional experimental data are required to determine whether biodegradation is important in determining the environmental fate of the compound. Bromophos undergoes significant photolysis in aqueous solution and on soil surfaces (Allmaier et al., 1984), and it is expected to photolyze in the ambient atmosphere. Hydrolysis of bromophos is rapid only under alkaline conditions approaching a pH of 9 (Aly and Badawy, 1982) and thus may not be significant in nature. Based on estimation methods that use the physical properties of bromophos, the compound is expected to be tightly bound to soil and to particulate organic matter and sediment in water; therefore, leaching is not expected to be significant. Bioaccumulation in aquatic organisms may be important. Soil persistence studies (Bro-Rasmussen et al., 1970; Eichler, 1972; Harris and Hitchon, 1970) have shown that bromophos will have a half-life of 1-7 weeks depending on soil type and conditions.

Environmental and human exposure to bromophos will most likely occur from its use as an insecticide. It has not been detected or monitored in ambient air or water. Bromophos has been detected in ready-to-eat foreign foods (Andersen, 1981, 1983; Cerna and Karmazin, 1981); however, it has not been monitored in ready-to-eat American foods. Food or food products stored with bromophos applications or foods stored in granaries whose surfaces were treated with bromophos may contain small residues of the insecticide.

Studies using rats indicate that bromophos is rapidly and nearly completely absorbed from the gastrointestinal tract, localized primarily in the liver and kidneys and not accumulated in any tissue (Stiasni et al., 1967). Metabolism occurred by hydrolyzing of the methyl phosphate or phenyl phosphate bond or both; phosphate was the primary urinary metabolite, and unchanged bromophos or its oxygen analog (bromoxon) were not detected. Elimination of metabolites occurred primarily through the urine (~96% in 24 hours) and was essentially complete after 48 hours. Transplacental distribution of bromophos has been observed in rats (Ackerman and Engst, 1970; Ackerman, 1974). Studies with ruminants indicate that dermal absorption of bromophos can occur (Dedek and Schwarz, 1969; Clark et al., 1966).

Bromophos has not been adequately tested for carcinogenicity. Administration of bromophos by gavage to groups of 25 male and 25 female rats at doses of 87.5, 175 or 350 mg/kg or to groups of three male and three female dogs at doses of 11, 43.8, 87.5 or 175 mg/kg each workday for up to 2 years produced no tumors or other evidence of neoplasia (Kinkel et al., 1965). These studies were not specifically designed for the assessment of carcinogenicity, however, and are limited by deficiencies that include poor survival (rats) and small group sizes (dogs). Bromophos was not mutagenic in reverse mutation assays with Salmonella typhimurium (Lippens et al., 1983), in a forward mutation assay with the yeast Schizosaccharomyces pombe (Gilot-Delhalle et al., 1983; Degraeve et al., 1980), in a recessive lethal mutation assay with Drosophila melanogaster (Benes and Sram, 1969) and in a dominant lethal mutation assay with intraperitoneally injected mice (Degraeve et al., 1980, 1984a). Intraperitoneal injection of bromophos also did not produce chromosomal aberrations in the bone marrow cells or spermatogonia of mice (Degraeve et al., 1984a,b).

Oral (gavage) administration of bromophos to rabbits on days 6-18 of gestation at dosages of 25, 50, 100, 200 or 400 mg/kg/day produced no increase in the type or incidence of fetal malformations (Leuschner and Leuschner, 1966a). Effects indicative of maternal toxicity (unspecified debilitation and limb flaccidity) and fetotoxicity (reduced number of full grown live fetuses) occurred, however, at doses >25 mg/kg/day. Administration of diets providing 5, 20 or 80 mg bromophos/kg bw/day to rats during a 3-generation reproductive study produced no malformations in any of the offspring (Leuschner et al., 1967). Although these results do not provide evidence that bromophos is teratogenic, it should be noted that the studies are unpublished and that endpoints and results were inadequately reported in a secondary source.

Treatment with 80 mg bromophos/kg bw/day in a 3-generation reproduction study, however, caused a high incidence (not specified) of stillbirths in the F_{1a} and subsequent generations and decreased neonatal survival in the parent and F_{1b} generations (Leuschner et al., 1967). Cholinesterase inhibition was the only other treatment-related effect found; erythrocyte cholinesterase inhibition occurred at 20 mg/kg/day, plasma and liver cholinesterase inhibition occurred at 5 mg/kg/day and brain cholinesterase inhibition occurred at 20 mg/kg/day (males) and 80 mg/kg/day (females) (Leuschner et al., 1967; Muacevic et al., 1970).

Administration of bromophos by gavage at doses of 11, 43.8, 87.5 or 175 mg/kg every workday for 2 years to groups of three male and three female dogs caused impaired spermatogenesis with proliferation of Leydig's cells in 2/3 high-dose male dogs, but had no effect on the "sexual cycle" of the females (Kinkel et al., 1965). In another study, seminiferous tubule degeneration occurred in 3/6 male dogs that received 175 mg/kg/day in capsules for 270 days (C.H. Boehringer Sohn, 1968), but not in groups of three dogs

exposed to 87.5 or 0 mg/kg/day. Prostate hypertrophy reportedly was more evident in the treated dogs, but additional information regarding the occurrence of this effect was not reported. Identical administration of bromophos to the same size groups of female dogs produced no histological alterations in the ovaries or uterus, but 2/6 dogs in the high-dose group were reportedly at no time in heat.

Gavage administration of bromophos to groups of 25 male and 25 female rats at dosages of 87.5, 175 or 350 mg/kg, 5 days/week for 2 years produced decreased body weight gain, proteinuria and hematuria at the high dose, and reduced plasma, erythrocyte, liver and brain cholinesterase at all dosages (Kinkel et al., 1965). In another study (Muacevic et al., 1970) of identical design except for group sizes (10 or 11/dose) plasma, liver and brain cholinesterase activities were strongly inhibited at all dosages, but erythrocyte cholinesterase was not markedly reduced at any dose. Daily administration of higher dosages of bromophos (188, 750 or 1250 mg/kg/day) by gavage to groups of 16 male rats for 100 days (Kinkel and Seume, 1963) produced similar effects (reduced body weight gain with lowered food consumption only at the high dose, and reduced plasma, liver and brain cholinesterase at all doses). Additionally, hydropic swelling of the liver cells occurred at all doses and hyaline droplets in the renal tubular cells and protein in the renal tubules occurred at the high dose. In another 100-day study, groups of six male and six female rats were maintained on diets that provided 0, 0.65, 1.25 and 2.5 mg bromophos/kg bw/day (Leuschner and Leuschner, 1966a). Statistically significant inhibition of plasma cholinesterase occurred in the high-dose female group, but activity returned to normal 2 weeks after cessation of treatment. Inhibition of plasma cholinesterase in the males and inhibition of erythrocyte cholinesterase in either sex were not produced in any of the treatment groups.

In a 2-year study, groups of three male and three female dogs were administered bromophos by gastric tube at dosages of 11, 43.8, 87.5 and 175 mg/kg on each workday (Kinkel et al., 1965). Treatment-related effects in the high-dose group included intoxication (respiratory difficulty, salivation, tremors, ataxia, hindlimb paresis) in 5/6 dogs that resulted in the deaths of three (sex unspecified); one dog in the 87.5 mg/kg group died after developing similar signs of toxicity. Small foci of ganglion cell degeneration in the spinal cord were observed in the dogs that died, but histologic alterations were not evident in the CNS or other unspecified tissues in any of the other treated dogs. Plasma, erythrocyte, liver and brain cholinesterase activities were depressed at all treatment levels. Treatment of similar numbers of dogs at the same dosage levels (0, 11, 43.8, 87.5 and 175 mg/kg/day, respectively) by capsule for 2 years caused inhibition of plasma and erythrocyte cholinesterase at all but the low dose, and inhibition of liver and brain cholinesterase at all treatment levels. Dogs treated with 87.5 or 175 mg bromophos/kg bw/day by capsule (three and six dogs/sex, respectively) for 270 days had depressed plasma and erythrocyte cholinesterase activities, but extensive neurohistological examinations revealed no treatment-related alterations (C.H. Boehringer Sohn, 1968). In a 100-day study, groups of 1, 2, 2 and 2 dogs of each sex received capsules that provided 0, 0.75, 1.5 and 3.0 mg bromophos/kg bw/day, respectively (Kinkel and Dirks, 1966). Slight equivocal decreases or no decreases in plasma or erythrocyte levels occurred throughout the study and 7 weeks postexposure, but liver and brain cholinesterase were normal when assayed 7 weeks postexposure.

The acute LC_{50} value for most fish is ~0.5 mg/l. Bromophos appears to be ~1-2 times more toxic to aquatic invertebrates and plants than to fish.

An ADI of 0.005 mg/kg/day or 0.35 mg/day for a 70 kg man was derived using the rat NOAEL of 5 mg/kg/day from the 3-generation reproduction study of Leuschner et al. (1967) and an uncertainty factor of 1000. This NOAEL was associated with inhibition of plasma and liver cholinesterase activity, while higher doses (20 mg/kg/day) were associated with brain cholinesterase inhibition which has known functional significance. The 20 mg/kg/day dose appeared to represent a LOAEL for males and a NOAEL for females. An RQ of 100 has been recommended based on brain cholinesterase inhibition observed in rats and dogs in the studies of Leuschner et al. (1967) and Kinkel et al. (1965). Because data were inadequate to assess the carcinogenicity of bromophos, this compound has been classified by IARC as a Group 3 and by U.S. EPA CAG as a Group D chemical.

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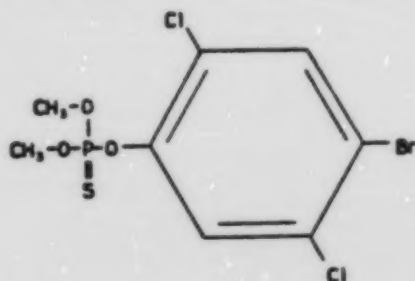
LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
BCF	Bioconcentration factor
bw	Body weight
CAS	Chemical Abstract Service
CNS	Central nervous system
CS	Composite score
EEG	Electroencephalogram
K _{oc}	Soil sorption coefficient standardized with respect to organic carbon
K _{ow}	Octanol/water partition coefficient
LC ₅₀	Concentration lethal to 50% of recipients (and all other subscripted dose levels)
LD ₅₀	Dose lethal to 50% of recipients
LOAEL	Lowest-observed-adverse-effect level
MAD	Minimum active dose
MED	Minimum effective dose
NOAEL	No-observed-adverse-effect level
NOEC	No-observed-effect concentration
NOEL	No-observed-effect level
RQ	Reportable quantity
RV _d	Dose-rating value
RV _e	Effect-rating value
WS	Water solubility

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Bromophos is the common name for O-(4-bromo-2,5-dichlorophenyl)O,O-dimethyl phosphorothioate. It is an insecticide known by the trade names Nexlon®, Omexan® and Brofene®. The structure, molecular weight and empirical formula for bromophos are as follows:



Molecular weight: 366.00

Molecular formula: $C_8H_8BrCl_2O_3PS$

The CAS Registry number for this compound is 2104-96-3.

1.2. PHYSICAL AND CHEMICAL PROPERTIES

Pure bromophos is a yellowish crystalline solid at room temperature that is soluble in most organic solvents, and has a faint characteristic odor (Martin and Worthing, 1977). Selected physical properties are listed below:

Melting point:	53-54°C	Worthing and Walker, 1983
Boiling point: at 0.01 mm Hg	140-142°C	Worthing and Walker, 1983
Density:	1.625 (60°C)	Hartley and Kidd, 1983
Vapor pressure: at 20°C	1.28×10^{-4} mm Hg	Worthing and Walker, 1983
Water solubility: at room temperature	40 ppm	Martin and Worthing, 1977
at 20°C	0.7 ppm	Worthing and Walker, 1983
at 20°C	0.30 ppm	Bowman and Sans, 1983a
Log K_{ow} :	4.884	Bowman and Sans, 1983a

A discrepancy exists in the reported values of water solubility, but neither Martin and Worthing (1977) nor Worthing and Walker (1983) report the source of their data. Bowman and Sans (1983a), however, experimentally determined water solubility and log K_{ow} values; the laboratory procedure is reported in Bowman and Sans (1983b). Therefore, the correct water solubility for bromophos at 20°C will be assumed to be 0.30 ppm, and this value will be used in predicting its environmental fate.

Bromophos is stable in aqueous suspension up to pH 9, but slowly hydrolyzes in aqueous alkaline media >pH 9 (Hartley and Kidd, 1983; Worthing and Walker, 1983). It is compatible with all pesticides except sulfur-containing compounds and organometallic compounds (Hartley and Kidd, 1983).

1.3. PRODUCTION DATA

Bromophos was introduced as an insecticide in 1964 by C.H. Boehringer Sohn/CELA GmbH (Martin and Worthing, 1977), which is the only manufacturer of the compound (Hartley and Kidd, 1983). Import figures for bromophos are not available.

Bromophos is manufactured by the reaction of O,O-dimethyl phosphorochloridothioate with 4-bromo-2,5-dichlorophenol (Martin and Worthing, 1977).

1.4. USE DATA

Bromophos is used as a broad range, non-systemic contact insecticide (Worthing and Walker, 1983; Hartley and Kidd, 1983) for protection of crops, forests and ornamental trees; control of flies, mosquitoes, storage insects and household pests; and as a seed dressing for vegetable seeds.

1.5. SUMMARY

Pure bromophos is a yellowish crystalline solid at room temperature that has a faint characteristic odor. It is almost insoluble in water, but is soluble in most organic solvents. Bromophos is used as a contact insecticide, but it is not manufactured in the United States.

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. WATER

2.1.1. Hydrolysis. Aly and Badawy (1982) experimentally investigated the hydrolysis of bromophos at 20°C over the pH range of 5-9. The hydrolysis was found to be pseudo-first-order with the following rate constants and half-lives:

<u>pH</u>	<u>Rate Constant (day⁻¹)</u>	<u>Half-life (days)</u>
5	0.00576	120.3
7	0.01266	54.7
9	0.4375	1.6

An increase in temperature of 10°C raised the reaction rate by 50%. These data indicate that bromophos is relatively stable in acidic and neutral media; however, increasing the pH significantly decreases the stability.

Under weak alkaline conditions, the hydrolytic product O-methyl-O-2,5-dichloro-4-bromophenyl thionophosphate is formed (Eichler, 1971). The hydrolytic decomposition products containing phosphorus are sensitive to further hydrolysis and the latter product forms the corresponding thionophosphate as an end product.

2.1.2. Oxidation. Pertinent data regarding the oxidation of bromophos could not be located in the available literature as cited in the Appendix.

2.1.3. Photolysis. The photolysis of bromophos in rainwater has been experimentally studied by Allmaier et al. (1984), Allmaier and Schmid (1985) and Fogy et al. (1983). Bromophos (80 ppm) was added to 250 g of rainwater (collected in Vienna, Austria) in open, wide-neck quartz bottles and subjected to 96 days of artificial sunlight irradiation for 12 hours, followed by 12 hours of darkness at a constant 27°C temperature. The irradiating

source produced no wavelength <290 nm. Under irradiating conditions, bromophos degraded rapidly with a half-life of 1.5 days. In the dark control, bromophos had a half-life of 5.8 days. The primary degradation product 4-bromo-2,5-dichlorophenol was found in both tests irregardless of the light conditions.

The above experiment, used to simulate the photolysis of bromophos under ambient aquatic conditions, indicates that photolytic degradation may be a major pathway for the removal of bromophos from the aquatic environment. The degradation in the experiment in the dark is probably due to hydrolysis, thermal decomposition or volatilization (Allmaier and Schmid, 1985). It is difficult to compare the degradation rate during darkness with hydrolytic degradation alone because the pH of the rainwater was not reported.

2.1.4. Microbial Degradation. In general, organophosphorus insecticides are capable of being metabolized by microorganisms because certain microorganisms can hydrolyze, reduce or oxidize these types of compounds (Lal, 1982). Data specifically for bromophos are limited. Stenersen (1969) isolated 50 strains of microorganisms from garden soil and examined their ability to degrade bromophos. All of the microorganisms degraded bromophos to some extent, but the fungi Alternaria tenuis and Trichoderma lignorum were especially capable of metabolizing bromophos rapidly. These fungi oxidize bromophos by a double dealkylation to bisdemethylated bromophos. Nestor (1972) found that bromophos, at concentrations of 5-100 µg/l, inhibited the growth of gram-positive bacteria, and to a lesser degree, that of gram-negative bacteria; bromophos was also found to have a bacteriostatic effect against Bacillus anthracis and enterococci.

While the data indicate that a few species of pure microorganisms can metabolize bromophos, the overall importance of biodegradation in the natural aquatic environment cannot be predicted without additional experimental data.

2.1.5. Volatilization. Calculation of Henry's Law Constant at 20°C from the vapor pressure (1.28×10^{-4} mm Hg) and WS (0.30 ppm) yields a value of 2.05×10^{-4} atm·m³/mol, suggesting that volatilization from aquatic environments is significant, although not rapid (Lyman et al., 1982).

2.1.6. Adsorption. The relatively low water solubility (0.30 ppm) and high log K_{ow} (4.884) suggest that adsorption to particulate organic matter and sediment in the aquatic environment may be important. It should be noted that bromophos tightly bound to aquatic particulate matter and sediment will not significantly volatilize, thereby diminishing the relative importance of volatilization from aquatic media.

2.1.7. Bioconcentration. Experimental aquatic bioconcentration data were not located in the available literature. The BCF can be estimated, however, from the log K_{ow} and WS by the following regression equations (Lyman et al., 1982):

$$\log BCF = 0.76 \log K_{ow} - 0.23 \quad (2-1)$$

$$\log BCF = 2.791 - 0.564 \log WS \text{ (in ppm)} \quad (2-2)$$

The BCF values calculated from Equations 2-1 and 2-2 are 3033 and 1219, respectively. Both values indicate that bioaccumulation in aquatic organisms is potentially significant.

2.2. AIR

Data specific to the environmental fate and transport of bromophos in the atmosphere were not located in the available literature. As noted in

Section 2.1.3., bromophos has been shown to undergo significant photodegradation in rainwater; therefore, it is likely that bromophos will undergo direct atmospheric photolysis.

2.3. SOIL

2.3.1. Microbial Degradation. Stenersen (1969) isolated 50 strains of microorganisms from garden soil and found these strains capable of degrading bromophos; two species of fungi were especially able to degrade bromophos (see Section 2.1.4.). This indicates that biodegradation of bromophos in soils may occur, but the relative importance of biodegradation is unclear. No study was available that examined degradation in natural soil and compared it with degradation in a sterilized sample of the same soil.

2.3.2. Chemical Degradation. Since bromophos undergoes significant aqueous hydrolysis under alkaline conditions (see Section 2.1.1.), it is possible that bromophos may hydrolyze in moist, alkaline soils.

2.3.3. Photolysis. Allmaier et al. (1984), Allmaier and Schmid (1985) and Fogy et al. (1983) have studied the photolysis of bromophos on soil surfaces under laboratory conditions. A concentration of 180 ppm bromophos was homogeneously mixed into a loamy sand soil and exposed to artificial sunlight (irradiation >290 nm) for 96 days. The soil water content was maintained at 12% and the temperature was maintained at 25°C. The soil was exposed to the artificial sunlight 12 hours/day, followed by 12 hours of darkness to simulate a day-night cycle. Repeat of the experiment under total darkness was used as a control. The half-life of bromophos during the day-night cycle was 48.3 days while the half-life during total darkness was 80 days, indicating that photodegradation of bromophos on certain soil surfaces is an important fate mechanism. 4-Bromo-2,5-dichlorophenol was found to be a degradation product in both the day-night test and the

control; this compound has also been found as a product of hydrolysis (Eichler, 1972). The degree of microbial degradation versus chemical degradation during these tests was not examined.

2.3.4. Leaching/Adsorption. Experimental data regarding the leaching of bromophos in soils were not located in the available literature. The K_{oc} may be estimated from the following regression equations (Lyman et al., 1982) which use the WS or $\log K_{ow}$ values:

$$\log K_{oc} = 3.64 - 0.55 \log WS \text{ (in ppm)} \quad (2-3)$$

$$\log K_{oc} = 0.544 \log K_{ow} + 1.377 \quad (2-4)$$

The K_{oc} values calculated from Equations 2-3 and 2-4 are 8464 and 10,812, respectively, based on a WS of 0.30 ppm and a $\log K_{ow}$ of 4.884. These K_{oc} values indicate that bromophos is expected to be relatively immobile in soil. Estimation of K_{oc} based on the molecular topology and quantitative structure analysis method of Sabljic (1984) yields a K_{oc} of ~33,750, which also indicates low soil mobility.

2.3.5. Volatilization. The relatively low vapor pressure (1.28×10^{-4} mm Hg) and high estimated soil adsorption of bromophos suggest that evaporation from soil is not an important fate mechanism.

2.3.6. Persistence. Bro-Rasmussen et al. (1970) examined the disappearance rate of bromophos in both laboratory and outdoor experiments. The laboratory experiments, conducted over an 80-day period, used a loam soil in 10 x plastic pails and an application rate of 10 kg/hectare; soil moisture was maintained at 75% field capacity. Degradation appeared to proceed in a linear fashion and an average half-life of ~72 days was observed. In the outdoor experiments, which used an application rate of 2 kg/hectare, a triphasic degradation was observed from the months of June through November. A half-life of 41 days was reported for the first phase, with ~20% of the initial concentration of bromophos remaining after 10 months.

Eichler (1972) studied the persistence of bromophos in three different soil types for a 26-week period following a single application rate of 5 kg/hectare. In both sandy (pH 6.5) and clay (pH 7.4) soil types, bromophos had a half-life of ~1 week with virtually no detectable residues after 9 weeks. In acidic (pH 5.2) high moorland soil having a high organic matter content, a half-life of ~7 weeks was observed; ~4% of the initial application amount remained after 26 weeks, and 4-bromo-2,5-dichlorophenol was found as a product of hydrolysis. The longer half-life in moorland soil was speculated to be due to strong sorption of the active ingredients by the soil particles, rendering the compound less vulnerable to chemical and physical degradation.

Harris and Hitchon (1970) used laboratory tests to examine the soil persistence and biological activity of bromophos. In a sandy loam soil, bromophos lost virtually all of its biological activity in ~4 weeks. Haefner (1983) reported that bromophos was relatively persistent in a sandy loam soil, with residues >1 ppm remaining 50 days after its application.

Insecticidal sprays of bromophos have been shown to persist from several weeks to 40 weeks when applied to concrete, metal, plywood, glass, hardwood, brick or plastic surfaces (Watters, 1976; Pawar and Yadav, 1980).

2.4. SUMMARY

In the aquatic environment, hydrolysis is not expected to be a major removal process for bromophos in either neutral or acidic waters (Aly and Badawy, 1982). Surface photolysis of bromophos is expected to be a significant fate mechanism based on laboratory tests (Allmaier et al., 1984). While bromophos appears to be degradable by microorganisms, the assessment of the relative importance of biodegradation requires additional experimental data. Based on the low water solubility and high K_{ow} value of

bromophos, the compound can be predicted to bioaccumulate in aquatic organisms and adsorb to particulate organic matter and sediments to a significant degree. Direct photolysis of bromophos in the atmosphere will probably occur.

In soil, bromophos is susceptible to photodegradation from the soil surface (Allmaier et al., 1984). Degradation may occur by microorganisms such as fungi (Stenersen, 1969), and chemical hydrolysis may occur in moist alkaline conditions. Estimation of the K_{oc} suggests that bromophos will not significantly leach from soil. Soil persistence studies (Bro-Rasmussen et al., 1970; Eichler, 1972; Harris and Hitchon, 1970) have shown that bromophos has a half-life of 1-7 weeks depending on soil type and conditions.

3. EXPOSURE

3.1. WATER

Bromophos is most likely to enter the aquatic environment from its direct use as an insecticide applied by aerial or ground spraying techniques or from direct application near bodies of water. Contamination of surface waters from runoff and of groundwater from leaching is also possible; however, bromophos is not expected to leach significantly in soil (see Section 2.3.4.). Since bromophos is not manufactured in the United States (see Section 1.3.), exposure from production facilities does not occur, but minor releases from formulation plant wastewaters are possible.

The U.S. EPA STORET Data Base contains no observations for bromophos. Additional monitoring data pertaining to the monitoring of bromophos in the aquatic environment could not be located in the available literature as cited in the Appendix.

3.2. FOOD

Bromophos is used as an insecticide in crop protection and storage; consequently, it has been detected in food products exposed to it. Bromophos has been detected in ready-to-eat foods in Denmark (Andersen, 1981, 1983), on oranges in Czechoslovakia (Cerna and Karmazin, 1981) and in essential oils of fruit in Italy (Leoni and D'Alessandro deLuca, 1978). Bromophos has not been monitored in ready-to-eat American foods.

The uptake and persistence of bromophos in bulk stored wheat in which the granary surfaces were treated with bromophos have been studied by Mensah and Walters (1979) and Mensah et al. (1979a,b). Bromophos residues were detected in the wheat after 7 months of storage; milled flour made from the treated wheat contained ~1 ppm bromophos. Similar results were obtained in wheat studies by Eichler and Knoll (1975); 0.1 ppm bromophos was detected in bread made from flour containing 0.8-1.2 ppm bromophos.

Eichler (1972) reported the results of field tests in which bromophos was applied to a variety of fruits and vegetables, and residues remaining were then monitored over a period of time. In general, residue levels were significantly <1 ppm and below permissible levels 2 weeks after application. Aslanyan et al. (1980) reported that bromophos residues persist 2-7 weeks after application to cabbage, apples, cotton, melons and grapes.

3.3. INHALATION

Bromophos probably enters the atmosphere during application procedures such as spraying, but ambient monitoring studies of bromophos in air were not located in the available literature.

Occupational inhalation exposure to bromophos inside an insecticide formulation plant has been reported (Davies, 1977).

3.4. DERMAL

Pertinent data regarding dermal exposure of humans to bromophos could not be located in the available literature; however, exposure resulting from insecticidal application or formulation of bromophos is very likely.

3.5. SUMMARY

Environmental exposure to bromophos is most likely to result from its use as an insecticide. It has not been detected or monitored in ambient water or air. Bromophos has been detected in ready-to-eat foreign foods (Andersen, 1981, 1983; Cerna and Karmazin, 1981) but ready-to-eat American foods have not been monitored. Food or food products stored with bromophos application or foods stored in granaries where surfaces were treated with bromophos may contain small residues of the insecticide.

4. PHARMACOKINETICS

4.1. ABSORPTION

Of the radioactivity from a 10 mg/kg oral dose of tritium-labeled bromophos, ~96% was excreted in the urine by male Wistar rats in 24 hours (Stiasni et al., 1967). These data indicate that the gastrointestinal absorption of bromophos was nearly complete.

Oral administration of 10 mg/kg ^{32}P -labeled bromophos to 10 male Wistar rats produced maximum levels of radioactivity in the blood after ~7 hours (Stiasni et al., 1967). The radioactivity was eliminated from the blood in a biphasic manner with a half-time for the B-phase of ~14 hours, corresponding to an elimination constant (K_2) of 0.05 hour^{-1} . An absorption constant (K_1) of 0.31 hour^{-1} and the time to maximum blood level of 7 hours were calculated from this value.

Studies with ruminants indicate that bromophos can be absorbed dermally. Radioactivity was detected in the blood of cows following the cutaneous (spray) application of 20 mg of ^{32}P -bromophos (Dedek and Schwarz, 1969); approximate concentrations of bromophos in the blood ranged from 0.5 ppm after 12 hours to 0.1 ppm after 48 hours. In another study, bromophos was detected in the omental fat of shorn lambs that were dipped in 0.5% bromophos (xylene-Triton 100 vehicle) once weekly for 9 weeks (Clark et al., 1966); residues averaged 9.75, 2.5 and 0.33 ppm at 1, 8 and 22 days, respectively, after the final dipping.

4.2. DISTRIBUTION

The analysis of radioactivity in organs following sacrifice of groups of two male Wistar rats, 6 and 12 hours after oral administration of 10 mg/kg ^{32}P -bromophos, showed relatively large proportions of the administered radioactivity in the liver (6.6 and 2.9%, respectively), kidney (2.5 and

0.9%, respectively), stomach/small intestine (4.6 and 2.3%, respectively) and large intestine (4.6 and 3.2%, respectively) (Stlasni et al., 1967). Oral administration of tritium-labeled bromophos (10 mg/kg) to two rats resulted in similar tissue distribution after 8 hours (1.6, 0.5, 21.3, 5.8, 4.5 and 0.3% of the administered radioactivity in the liver, kidney, stomach, small intestine, large intestine and heart, respectively). Radioactivity levels in mesenteric fat and muscles were $\leq 0.1\%$ in all experiments. Bromophos showed no tendency to accumulate in any tissue, as elimination from the urine and feces was essentially complete after 24 hours (Section 4.4.).

Transplacental distribution of bromophos can occur. Bromophos was detected in the placenta, fetal brain, liver and muscles, as well as maternal liver, 30 minutes after the oral administration of 20 mg to rats (Ackerman and Engst, 1970; Ackerman, 1974). Traces of bromoxon metabolite were detected in the fetal muscle, but additional information regarding these studies was not reported.

4.3. METABOLISM

Bromophos is metabolized by hydrolysis of the methyl phosphate or the phenyl phosphate bond or both (Stlasni et al., 1967). Five metabolites were detected in the urine of rats after the oral administration of 10 mg/kg ^{32}P -bromophos, and three were detected in rats following the oral administration of an unspecified dose of thymidine-labeled bromophos. Analyses showed that the metabolites were phosphate, dimethylthionophosphate, monodesmethylbromophos and dichlorobromophenol; two metabolites were not identified. Unmetabolized bromophos, its oxygen analog bromoxon and monodesmethylbromoxon were not found in the urine. Phosphate accounted for ~5, 20

and 60% of the metabolites of the ^{32}P -bromophos dose after 1.5, 24 and 48 hours, respectively, but the excretion of dimethylthionophosphate and monodesmethylbromophos remained constant (~9 and 14%, respectively).

A more recent study reported that the excretion of O,O-dimethylthiophosphate by rats was independent of dose or route (Riemer et al., 1985). Bromophos was administered at oral or intraperitoneal doses of 3, 30 or 300 mg/kg.

4.4. EXCRETION

In three groups of two rats, ~96 and 1-3% of the radioactivity from oral doses of 10 mg/kg of tritium-labeled bromophos were eliminated in the urine and feces, respectively, in 24 hours (Stiasni et al., 1967). Elimination was essentially complete in 48 hours. Identically designed experiments with ^{32}P -bromophos showed similar patterns of elimination, but elimination of radioactivity in the urine and feces totaled ~63-64% and 14-16% of the administered amounts, respectively, in 48 hours. The lower urinary values were attributed to incorporation of cleaved phosphate into the phosphate pool of the rats, and the relatively high ^{32}P content in the feces was attributed to metabolism of bromophos in the intestine.

Biliary elimination experiments were also conducted by Stiasni et al. (1967). Groups of three rats were administered 10 mg/kg of ^{32}P -bromophos or 5 mg/kg of tritium-labeled bromophos intraduodenally. Only ~1% of the ^{32}P activity was detected in the bile during 8 hours following administration, but ~25% of the tritium activity was excreted within the same period. These studies indicate that neither unchanged bromophos nor its phosphorus-containing metabolites were excreted in the bile, and that the tritium activity in the bile is attributable to dichlorobromophenol and its

metabolites. Biliary-excreted tritium is probably resorbed from the intestine by enterohepatic recirculation since only ~2% of oral doses were found in the feces.

4.5. SUMMARY

Orally-administered bromophos was absorbed rapidly and nearly completely by rats, localized primarily in the liver and kidneys and not accumulated in any tissue (Stiasni et al., 1967). Metabolism occurred by hydrolyzing the methyl phosphate or phenyl phosphate bond or both; phosphate was the primary urinary metabolite, and unchanged bromophos or its oxygen analog (bromoxon) were not detected. Elimination of metabolites occurred primarily through the urine (~96% in 24 hours) and was essentially complete after 48 hours.

Transplacental distribution of bromophos has been observed in rats (Ackerman and Engst, 1970; Ackerman, 1974). Studies with ruminants indicate that dermal absorption of bromophos can occur (Dedek and Schwarz, 1969; Clark et al., 1966).

5. EFFECTS

5.1. CARCINOGENICITY

A summary of an unpublished study reported that groups of 6-month-old rats were administered bromophos by gavage at doses of 0, 87.5, 175 and 350 mg/kg each workday for 2 years (Kinkel et al., 1965). The strain, chemical purity and vehicle were not specified. The groups consisted of 25 male and 25 female rats but because of poor survival, 3, 5, 5 and 17 rats were added to the test after the start (not elaborated) in the 0, 87.5, 175 and 350 mg/kg groups, respectively. One male and one female rat apparently from each treatment group, were sacrificed after 8, 10, 12, 15, 18 and 21 months for gross and histopathological examinations; it was implied that the remaining rats were examined following sacrifice in extremis, following death during the study, or at terminal sacrifice. Tumors did not occur in the control or treated groups, but the extent of the pathological examinations was not specified. Survival was poor, however, with only 5, 4, 2 and 5 males and 9, 6, 7 and 8 females surviving for 100 weeks in the 0, 98.5, 175 and 350 mg/kg groups, respectively; the poor survival appears to be attributable to the age of the rats at the start of the experiment. The effects of treatment on indices of toxicity other than neoplasia are discussed in Section 5.5.

Neoplasia also was not observed in groups of three male and three female mongrel dogs that were treated with bromophos by gavage at dosages of 11, 43.8, 87.5 or 175 mg/kg on each workday for 2 years (Kinkel et al., 1965) (Section 5.5.).

5.2. MUTAGENICITY

In the Ames reverse mutation assay with S. typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 (Lippens et al., 1983) and in a forward

mutation assay with the yeast Schizosaccharomyces pombe strain SP-198 (Gillot-Delhalle et al., 1983; Degraeve et al., 1980), bromophos yielded negative results. These assays were conducted with and without S-9 activation preparations, but were not reported in detail. Bromophos did not cause recessive lethal mutations in Drosophila melanogaster (Benes and Sram, 1969). Intraperitoneal injection of 1 g/kg bromophos into male Q strain mice did not produce dominant lethal mutations (Degraeve et al., 1980, 1984a) or chromosomal aberrations in the bone marrow cells or spermatogonia (Degraeve et al., 1984a,b). Details of the above assays are summarized in Table 5-1.

5.3. TERATOGENICITY

The design and results of an unpublished teratogenicity study (Leuschner and Leuschner, 1966a) were summarized by WHO/FAO (1973). Five groups of 10 pregnant rabbits of unspecified strain were administered 25, 50, 100, 200 or 400 mg bromophos/kg bw/day by gavage on days 6-18 of gestation. Forty rabbits served as controls, but the vehicle was not indicated. Fetuses were removed and examined on day 30 of gestation. No maternal toxic effects or fetotoxicity occurred in the 25 mg/kg/day treatment group, but maternal toxicity (unspecified debilitation, limb flaccidity, diarrhea) and fetotoxicity (reduced number of full grown live fetuses) were observed at the higher dosages. The type or incidence of fetal malformations in any of the treatment groups did not differ from that in the control group. Quantitative data or additional information regarding indices examined or results of this study were not reported.

Administration of diets that provided 5, 20 or 80 mg/kg/day bromophos to rats during a 3-generation reproduction study did not produce malformations

TABLE 5-1
Mutagenicity Testing of Bromophos

Assay	Indicator/Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	<u>Salmonella typhimurium</u> TA1535 TA1537 TA1538 TA98 TA100	NR	NR	<25 µg/plate	S-9 (rat, Aroclor)	- - - - -	Doses not reported, but toxicity evident at ≥25 µg/plate.	Lippens et al., 1983
Forward mutation	<u>Schizosaccharomyces pombe</u> SP-198	>99%	NR	NR	S-9 (mouse, phenobarbital)	-	Mutation at adenine loci scored. Negative data were inadequately reported.	Gilot-Delhalle et al., 1983; Degraeve et al., 1980
Recessive lethal	<u>Drosophila melanogaster</u>	NR	Intraperitoneal injection	0.2 µl of 0.2% water solution	NA	-	No comment.	Benes and Sram, 1969
Dominant lethal	mouse, ♀ strain, male	>99%	Intraperitoneal injection	1 g/kg (peanut oil suspension)	NA	-	Increased preimplantation loss and total fetal mortality during first week after treatment, but effects not significant (p values not reported) and not apparent during post-treatment weeks 2-7. No effect on frequencies of corpora lutea, implants, live embryos or postimplantation losses.	Degraeve et al., 1980, 1984a
Chromosome aberrations	mouse, ♀ strain, male, bone marrow cells and spermatogonia	>99%	Intraperitoneal injection	1 g/kg (peanut oil suspension)	NA	-	Percentage of fragments increased in spermatogonia, but not statistically significant (p values not reported)	Degraeve et al., 1984a,b

NA = Not applicable; NR = not reported

In any of the offspring (Leuschner et al., 1967). The extent of teratological examination was not specified, but additional information regarding the experimental design of this study is presented in Section 5.4.

5.4. OTHER REPRODUCTIVE EFFECTS

The results of an unpublished, reportedly "standard" 3-generation reproduction study (Leuschner et al., 1967) were summarized by WHO/FAO (1973) and Clegg (1979). Groups of 20 male and 20 female rats (strain unspecified) that were fed diets providing 0, 5, 20 and 80 mg/kg/day bromophos from weaning constitute the parent generation. These dose levels were maintained throughout all generations, but the size of the parental groups in the subsequent generations or numbers of litters or offspring were not specified. Treatment at 80 mg/kg/day caused decreased body weight gain (particularly in the males) and a high incidence of stillbirths in the F_{1a} and subsequent generations. The survival rate of the young was also reduced except in the first litter (presumably the F_{1a} generation) and the parent (F_0) generation. Appearance, behavior, food consumption, unspecified hematological parameters, fertility, and size and weight of the litters were unaffected by bromophos treatment. The rats in the 5 and 20 mg/kg/day treatment groups did not differ from control animals in any of the above parameters. Additional information regarding this study (i.e., indices examined or quantitative results) was not reported in the available summaries.

Cholinesterase activities were also investigated in the Leuschner et al. (1967) 3-generation reproduction study, but the details in the WHO/FAO (1973) summary were limited. The details and results of what appear to be the same assays, however, were reported by Muacevic et al. (1970). Determinations were made on 10 male and 10 female rats at each dosage level in the

parent (after 6 months of treatment) and F_1 and F_2 (after 8 months of treatment) generations. Cholinesterase inhibition in the plasma and liver occurred at 5 mg/kg/day, in the erythrocytes at 20 mg/kg/day (no-effect levels interpolated by an unstated method to be 15.5 mg/kg/day for males and 10.5 mg/kg/day for females) and in the brain at 20 mg/kg/day (males) and 80 mg/kg/day (females). Additional data were not reported, but the results of the filial generations' assays were identical to those of the parent generation.

In a summary of an unpublished study, WHO/FAO (1973) reported that "spermatogenesis was impaired with proliferation of Leydig's cells" in 2/3 male mongrel dogs administered 175 mg/kg bromophos by gavage every workday for 2 years (Kinkel et al., 1965). Spermatogenesis was normal in groups of three dogs that were exposed to 11, 43.8 or 87.5 mg/kg bromophos. Identical administration of bromophos to groups of three female dogs had no effect on the "sexual cycle." Nonreproductive effects of exposure in this study are detailed in Section 5.5. The dogs were 1-3 years of age and weighed 3.9-10.3 kg at the beginning of exposure.

In another unpublished study, C.H. Boehringer Sohn (1968) administered bromophos in capsules at doses of 0, 87.5 or 175 mg/kg bw/day to groups of 3, 3 and 6 mongrel dogs/sex, respectively, for 270 days. The dogs weighed 6-20 kg at the start; age was not specified. Histological examinations of the reproductive tracts showed seminiferous tubule degeneration in 3/6 male dogs that received 175 mg/kg/day and prostatic hypertrophy that was more evident in the treated dogs (not elaborated in the available summary). Histopathological alterations were not observed in the ovaries or uterus, but 2/6 high-dose females were reportedly at no time in heat.

5.5. CHRONIC AND SUBCHRONIC TOXICITY

Chronic and subchronic toxicity studies of bromophos have been conducted in both rats and dogs, but the preponderance of studies are unpublished and are available only as summaries (WHO/FAO, 1973). Treatment information was usually adequately reported, but there were deficiencies regarding other aspects of experimental design (i.e., indices examined) as well as results (i.e., lack of quantitation). The apparently incomplete nature of some of the following summaries, therefore, reflects the deficiencies of the available information. One of the published reports (Muacevic et al., 1970) details studies that are very similar or identical in design or results (or both) to several of the unpublished studies (Kinkel et al., 1965; Kinkel and Dirks, 1966; Leuschner et al., 1967). Since accounts of the unpublished studies are available only from the secondary source (WHO/FAO, 1973) and the same group of investigators were involved in both the unpublished and published studies, the possibility that some of these studies are the same cannot be precluded.

In an unpublished study, bromophos was administered by gavage at doses of 0, 87.5, 175 and 350 mg/kg to 6-month-old rats on each workday for 2 years (Kinkel et al., 1965). Groups consisted of 25 male and 25 female rats but 3, 5, 5 and 17 rats were added to the test after the start (not elaborated) in the 0, 87.5, 175 and 350 mg/kg groups, respectively. The strain of rats and vehicle were not specified. As detailed in Section 5.1., survival was poor in both the treated and control groups. Treatment-related effects included reduced body weight gain, proteinuria and hematuria in the high-dose group. At all treatment levels, cholinesterase inhibition occurred in the plasma, erythrocytes, brain and liver but the decreases were not quantified in the available summary. Behavior, unspecified hematological and liver function indices, and urinary ascorbic acid excretion were

unaffected by bromophos treatment. Gross and histological examinations of the kidneys and other unspecified organs conducted according to the schedule detailed in Section 5.1. did not reveal treatment-related lesions.

Cholinesterase activities were also determined in another study by the same group of investigators (Muacevic et al., 1970). Similarities in design and results suggest that this study may be the same as the Kinkel et al. (1965) study, but the limitations of the WHO/FAO (1973) summary of the Kinkel et al. (1965) study preclude unequivocal determination. Erythrocyte and plasma cholinesterase levels were determined 16, 20 and 24 months after the beginning of gavage administration of 0, 87.5, 175 and 350 mg/kg/day bromophos to groups of 10 or 11 FW-49 male and female rats. Additionally, cholinesterase activities in the brain and liver of the same rats were determined after 24 months. Mulgafarin® (3%; cellulose methyl ether) was used in each treatment as an emulsifier, and the control group received 3% Mulgafarin® in water. Marked reductions in cholinesterase activity in the plasma, brain and liver resulted after 24 months of treatment, but erythrocyte cholinesterase was not strongly inhibited. Cholinesterase inhibition in the erythrocyte, plasma, brain and liver ranged from 23-47%, 82-84%, 62-79% and 73-79% of control values, respectively. In the treatment groups, with no marked differences between the treatment groups or clear dose-related responses. Data variance precluded statistical analysis of the data, but erythrocyte and plasma cholinesterase activity at 16 and 20 months was generally comparable to the 24-month values at all doses.

In an unpublished study of shorter duration, groups of 16 male rats of unspecified strain were administered bromophos by gastric tube at doses of 0, 188, 750 and 1250 mg/kg/day for 100 days (Kinkel and Seume, 1963). Body weight gain was reduced in all the test groups with lowered food consumption

only at the highest dose. At all dose levels, brain, liver and plasma cholinesterase were inhibited, and dose-related hydropic swelling of the hepatic cells occurred. Hyaline droplets in tubular cells and protein in the tubules were observed in the kidneys at 1250 mg/kg, but unspecified urinalysis was unremarkable. The bromosulfophthalein retention test for liver function, unspecified hematological indices and gross appearance of unspecified organs were also normal.

In another 100-day study, groups of six male and six female Wistar rats were maintained on diets that provided 0, 0.65, 1.25 and 2.5 mg/kg bw/day bromophos (Muacevic et al., 1970; Leuschner and Leuschner, 1966b). The dietary concentrations and purity of the bromophos were not indicated. Erythrocyte and plasma cholinesterase activities were determined 1, 2, 4, 6, 8, 10, 12 and 14 weeks after administration. Brain cholinesterase activity apparently was not measured. Statistically significant ($p \leq 0.01$) inhibition of erythrocyte cholinesterase in either sex or plasma cholinesterase in the males was not produced in any of the treatment groups. A significant loss of plasma cholinesterase activity occurred in the 2.5 mg/kg/day females at weeks 4, 8, 10, 12 and 14, but not at the lower dosages; activity returned to control levels 2 weeks after cessation of treatment. It was concluded that the inhibitory threshold for plasma cholinesterase in the females ranged from 1.25-2.5 mg bromophos/kg bw. Behavior, growth, food intake and gross appearance of unspecified organs were reported to be normal in the treated rats (Leuschner and Leuschner, 1966b).

Desl et al. (1971) administered bromophos in the diet to groups of eight female albino rats at concentrations of 0, 10, 30, 100 and 500 ppm for 42 days. The diets reportedly provided 0, 1, 3, 10 and 50 mg bromophos/kg

bw/day. Cholinesterase activity was significantly reduced in the erythrocytes at 100 and 500 ppm ($p < 0.001$), in the plasma at 500 ppm ($p < 0.001$), and in the brain at 500 ppm ($p < 0.01$). Treatment-related effects on performance were not evident in a labyrinth test, and an unspecified number of rats used in this test also showed no treatment-related decrements in an orientation reaction test. Body weights were unaffected by treatment, but liver, kidney and dried cerebral weights were significantly increased in the 500 ppm group; adrenal and whole brain wet weights were not significantly affected by exposure. Pooled results of histological examinations of the livers, kidneys and brains from these rats and groups of 16 other rats that were exposed only to 100 and 500 ppm bromophos indicated that effects were limited to mild to moderate granular cell degeneration in the livers (three animals) and swelling of convoluted tubule epithelial cells in the kidneys (incidence not indicated) at 500 ppm. Dose-related performance decrements occurred in a conditioned reflex test in a separate, but identically designed experiment. In a third experiment, groups of 20, 16 and 16 rats were exposed to 0, 100 and 500 ppm bromophos in the diet, respectively, for 42 days, and a significant reduction in brain activity, as indicated by EEG monitoring, occurred at 500 ppm.

An abstract of a Russian study (Aslanyan, 1981) reported that bromophos was orally administered to white rats at dosages of 28 and 70 mg/kg for 4 months and 0.2-10 mg/kg for 9 months. Impaired excretory function of the kidney was reported at the high dosages; alterations included diuresis towards the end of treatment and impaired nitrogen and urea clearance. Kidney weights were increased in all treated rats. "Significant changes" in serum cholinesterase occurred at all dosages. Additional information regarding the design or results of this study were not reported.

In an unpublished 2-year study, groups of three male and three female mongrel dogs were administered bromophos (purity not specified) by gastric tube at dosages of 0, 11, 43.8, 87.5 and 175 mg/kg on each workday (Kinkel et al., 1965). The dogs were 1-3 years old and weighed from 3.9-10.3 kg. In the 87.5 and 175 mg/kg groups, 1/6 and 3/6 dogs died, respectively, after developing typical signs of organophosphorus insecticide poisoning (unspecified respiratory difficulty, salivation and tremors, followed by ataxia and paresis of the hindlimbs). Two additional dogs in the 175 mg/kg group developed the same effects but recovered, one after being taken off treatment and the other while administration was continued. Histological examination showed small foci of ganglion cell degeneration in the spinal cord of the dogs that died in the 87.5 and 175 mg/kg groups, but significant alterations in the CNS or other unspecified tissues were not observed in any of the other treated dogs. Relative organ weights and gross pathology were unremarkable in the 11 and 43.8 mg/kg group and, presumably, in the survivors in the 87.5 and 175 mg/kg groups. Plasma, erythrocyte, liver and brain cholinesterase activities were depressed at all dosages. Food intake, growth, urinary ascorbic acid excretion, unspecified hematological indices, liver function and unspecified urine tests were similar in treated and control groups.

The results of erythrocyte and plasma cholinesterase determinations in a similarly designed study by the same group of investigators were reported by Muacevic et al. (1970). In this study, groups of 1, 5, 5, 6 and 6 dogs of either sex (distribution not specified) were treated daily at dosages of 175, 87.5, 43.8, 11 and 0 mg bromophos/kg bw for 2 years. The bromophos was administered as a suspension mixed with 3% Mulgafarin® (cellulose methyl ether emulsifying agent) in gelatin capsules. After 24 months of administration, marked inhibition of cholinesterase occurred in the erythrocytes at

all but the low dose (98-99% inhibition vs. 32% in the low dose), in plasma at all but the low dose (70-81% inhibition vs. 27% in the low dose), and at all doses in the brain (57-65% inhibition) and liver (83-97% inhibition). Erythrocyte and plasma cholinesterase activities were also assessed after 16 and 20 months of exposure with similar results. Erythrocyte, plasma, brain and liver cholinesterase inhibition, with the exception of the erythrocyte and plasma values for the low dose, did not vary significantly with dose. It should be emphasized, however, that data variance precluded statistical analysis.

Mongrel dogs were also exposed to bromophos orally at dosages of 0 mg/kg/day (3 dogs/sex), 87.5 mg/kg/day (3 dogs/sex) and 175 mg/kg/day (6 dogs/sex) for 270 days (C.H. Boehringer Sohn, 1968). The compound was administered in capsules and the dogs initially weighed 6-20 kg. Dose-related occurrence of reduced body weight and food consumption was reported. Plasma and erythrocyte cholinesterase was inhibited (not quantitated) in both treated groups, but the results of unspecified hematological tests, serum analysis and urinalysis were normal. Extensive neuropathological examinations of the CNS, spinal ganglia and peripheral nerves failed to confirm the results (i.e., ganglion cell degeneration) of the Kinkel et al. (1965) study. Reproductive tract alterations were observed in some of the treated dogs (see Section 5.4.), but it was not indicated if other tissues were examined histologically.

In a 100-day study with beagle dogs, groups of two males and two females received diets (gelatin capsules) that provided 0.75, 1.5 and 3.0 mg bromophos/kg/day (Muacevic et al., 1970; Kinkel and Dirks, 1966). A control group consisted of one male and one female. The ages of the dogs were not specified, but initial weights ranged from 7.7-12.6 kg. Erythrocyte and

plasma cholinesterase activities were determined 4 weeks before administration (twice weekly) and weekly during the tests. One week after the final administration, half of the dogs from each group were sacrificed for determination of brain and liver cholinesterase activities. Erythrocyte and plasma cholinesterase activities were determined in the remaining dogs weekly for 7 weeks following exposure, and brain and liver cholinesterase activities were determined at the end of the 7-week postexposure period. Erythrocyte and plasma cholinesterase activity essentially ranged from 10-30% of the control values after the first several weeks of exposure; however, these values should not be regarded as indicative of slight inhibition because of the inadequate number of samples and variability of the data with regard to treatment week as well as dose. Additionally, a cholinesterase inhibition of <20% was regarded as normal. The erythrocyte and plasma cholinesterase levels were similar during the 7-week recovery period, and brain and liver cholinesterase were not inhibited (data not reported).

Workers exposed to bromophos during its production and formulation for 2 years apparently showed no alterations in erythrocyte cholinesterase levels, as indicated by monthly determinations (C.H. Boehringer Sohn, 1966). Additional information regarding this unpublished study was not reported in the available summary (WHO/FAO, 1973).

The results of a WHO Insecticide Testing Unit field evaluation of bromophos in Nigeria in 1964-1965 were summarized in WHO (1967). In one trial, eight workers sprayed a village with bromophos for 4 hours on 1 day and 2.5 hours on the following day. In a second trial, five workers sprayed three villages with bromophos for 14 days during a period of 22 days (spraying time ~4 hours/day). Protective clothing and masks were worn, but monitoring data were not reported. Clinical symptoms did not occur in any of the

sprayers, but plasma cholinesterase levels were slightly depressed in both groups. The lowest activity was 75% of the pre-exposure level, and levels returned to normal after 1 month. Additional data were not reported in the available summary of this study.

5.6. OTHER RELEVANT INFORMATION

Acute oral LD_{50} s of 1600 and 1730 mg/kg have been determined for male and female Sherman rats, respectively (Gaines, 1969). Dermal LD_{50} s in male or female Sherman rats were determined to be >5000 mg/kg. Technical grade bromophos, administered in peanut oil (oral) or xylene (dermal) was used in these studies.

Muacevic et al. (1970) found that maximum inhibition of erythrocyte and plasma cholinesterase activity in rats occurred 8-24 hours after the administration of a single oral dose of 0.5 LD_{50} (dose not specified). Normal cholinesterase activity ($\leq 20\%$ inhibition) in the plasma and erythrocytes returned after ~3 and 5 days, respectively. The authors noted that these results are consistent with the pharmacokinetic data of Stiasni et al. (1967) that showed that peak blood levels of bromophos in rats occurred 7 hours after oral administration.

An abstract of a Russian study (Sasinovich et al., 1977) reported that repeated topical application of bromophos (20 times at 100 mg/kg, schedule not specified) to rats, rabbits and guinea pigs disrupted liver function, as indicated by changes (unspecified) in serum proteins. Additional information regarding the design or results of this study was not reported.

Neurotoxicity in hens was reported in several unpublished studies, available only as summaries (WHO/FAO, 1973). Byewater (1966) reported severe demyelination in hens receiving 10 mg/kg/day of bromophos administered with atropine and 2-PAM. Ataxia and incoordination were observed in

all the birds and three died. No other details were reported. Kinkel and Hübner (1966) histologically evaluated the brain and spinal cord of 10 hens receiving 1 g/kg/day bromophos. Treatment was discontinued when signs of paralysis developed (between 12 and 56 days). They reported degeneration of ganglia cells and demyelination; however, their histological interpretation was questioned by Glees (1966). No other details of these studies were reported.

Baines (1966) found no delayed neurotoxic effects in two hens given a total of 5.5 g/kg bromophos in doses of 300 or 400 mg/kg over a 7-week period. Muacevic and Glees (1967) reported no degeneration in CNS or peripheral nerves in 10 hens observed for 3 weeks following two oral doses of 2 g/kg bromophos given at 3-week intervals. Muacevic and Glees (1968) also reported clinical neurological effects in 10 hens administered 12.5 or 125 mg/kg/day bromophos for 4 weeks. However, these effects disappeared upon cessation of treatment and no CNS alterations were observed in a neuropathological examination of two of the birds. No other details were reported.

5.7. SUMMARY

Neoplastic or preneoplastic lesions were not associated with the gavage administration of bromophos in unpublished long-term studies using rats or dogs (Kinkel et al., 1965). Dosages of 87.5, 175 or 350 mg/kg (rats) and 11, 43.8, 87.5 and 175 mg/kg (dogs) were administered on each workday for 2 years. It should be noted that the extent of gross and histological examinations was not indicated in the available summary of this study, that survival was poor in the rat study and that only three animals of each sex/dose were treated in the dog study.

The mutagenic/clastogenic potential of bromophos has been assessed in a limited number of studies with negative results. Bromophos was not mutagenic in reverse mutation assays with five strains of Salmonella typhimurium bacteria (Lippens et al., 1983) and in a forward mutation assay with the yeast, Schizosaccharomyces pombe strain SP-198 (Gilot-Delhalle et al., 1983; Degraeve et al., 1980) with or without S-9 activation preparations. Bromophos did not cause recessive lethal mutations in Drosophila melanogaster (Benes and Sram, 1969) or dominant lethal mutations (Degraeve et al., 1980, 1984a) or chromosomal aberrations in the bone marrow or spermatogonia (Degraeve et al., 1984a,b) when injected intraperitoneally into male Q strain mice.

Oral (gavage) administration of bromophos to rabbits on days 6-18 of gestation at daily dosages of 25, 50, 100, 200 or 400 mg/kg/day did not produce an increase in the type or incidence of fetal malformations (Leuschner and Leuschner, 1966a). Effects indicative of maternal toxicity (i.e., unspecified debilitation and limb flaccidity) and fetotoxicity (i.e., reduced number of full grown live fetuses) occurred, however, at doses ≥ 50 mg/kg/day. Administration of diets providing 5, 20 or 80 mg bromophos/kg bw/day to rats during a 3-generation reproduction study did not produce malformations in any of the offspring (Leuschner et al., 1967). Although these results provide evidence that bromophos is not teratogenic, it should be noted that the studies are unpublished and that endpoints and results were reported inadequately in a secondary source.

Treatment with 80 mg bromophos/kg bw/day in a 3-generation reproduction study in rats did cause a high incidence (not specified) of stillbirths in the F_{1a} and subsequent generations, and decreased neonatal survival in the parent and F_{1b} generation (Leuschner et al., 1967). Cholinesterase

inhibition was the only other treatment-related effect found; erythrocyte cholinesterase inhibition occurred at 20 mg/kg/day, plasma and liver cholinesterase inhibition occurred at 5 mg/kg/day and brain cholinesterase inhibition occurred at 20 mg/kg/day (males) and 80 mg/kg/day (females) (Leuschner et al., 1967; Muacevic et al., 1970). The cholinesterase determinations were made in the parent generation after 6 months of treatment and in the filial generation after 8 months of treatment; the results of the parent and filial assays were identical. Administration of bromophos by gavage at doses of 11, 43.8, 87.5 or 175 mg/kg every workday for 2 years to groups of 3 male and 3 female dogs caused impaired spermatogenesis with proliferation of Leydig's cells in 2/3 high-dose male dogs, but had no effect on the "sexual cycle" of the females (Kinkel et al., 1965).

In another study, seminiferous tubule degeneration occurred in 3/6 male dogs that received 175 mg/kg/day bromophos in capsules for 270 days (C.H. Boehringer Sohn, 1968), but not in groups of 3 dogs exposed to 87.5 or 0 mg/kg/day. Prostatic hypertrophy reportedly was more evident in the treated dogs, but additional information regarding the occurrence of this effect was not reported. Identical administration of bromophos to the same size groups of female dogs did not produce histological alterations in the ovaries or uteri, but 2/6 dogs in the high-dose group reportedly were at no time in heat.

Gavage administration of bromophos to groups of 25 male and 25 female rats at dosages of 87.5, 175 or 350 mg/kg, 5 days/week for 2 years produced decreased body weight gains, proteinuria and hematuria at the high dose, and reduced plasma, erythrocyte, liver and brain cholinesterase at all dosages (Kinkel et al., 1965). In another study of identical design, except for group sizes (10 or 11/dose), by the same group of investigators (Muacevic et

al., 1970), plasma, liver and brain cholinesterase activities were strongly inhibited at all dosages, but erythrocyte cholinesterase was not markedly reduced at any dose. Daily gavage administration of higher dosages of bromophos (188, 750 or 1250 mg/kg/day) to groups of 16 male rats for 100 days (Kinkel and Seume, 1963) produced similar effects (reduced body weight gain with lowered food consumption only at the high dose, reduced plasma, liver and brain cholinesterase at all doses). Additionally, hydropic swelling of the liver cells occurred at all doses, and hyaline droplets in the renal tubular cells and protein in the renal tubules were observed at the high dose. In another 100-day study, groups of six male and six female rats were maintained on diets that provided 0, 0.65, 1.25 and 2.5 mg bromophos/kg bw/day (Muacevic et al., 1970; Leuschner and Leuschner, 1966b). Statistically significant inhibition of plasma cholinesterase occurred in the high-dose female group, but activity returned to normal 2 weeks after cessation of treatment. Inhibition of plasma cholinesterase in the males and inhibition of erythrocyte cholinesterase in either sex were not produced in any of the treatment groups.

In a 2-year study with dogs, groups of 3 males and 3 females were administered bromophos by gastric tube at dosages of 11, 43.8, 87.5 and 175 mg/kg on each workday (Kinkel et al., 1965). Treatment-related effects in the high-dose group included intoxication (respiratory difficulty, salivation, tremors, ataxia, hindlimb paresis) in 5/6 dogs that resulted in the deaths of 3 (sex unspecified); one dog in the 87.5 mg/kg group died after developing similar signs. Small foci of ganglion cell degeneration in the spinal cord were observed in the dogs that died, but histological alterations were not evident in the CNS or other unspecified tissues in any of the other

treated dogs. Plasma, erythrocyte, liver and brain cholinesterase activities were depressed at all treatment levels. Treatment of 1, 5, 5, 6 and 6 dogs at the same dosage levels (175, 87.5, 43.8, 11 and 0 mg/kg/day, respectively) by capsule for 2 years caused inhibition of plasma and erythrocyte cholinesterase at all but the low dose and inhibition of liver and brain cholinesterase at all treatment levels. Dogs treated with 87.5 or 175 mg bromophos/kg bw/day by capsule (3 and 6 dogs/sex, respectively) for 270 days had depressed plasma and erythrocyte cholinesterase activities, but unlike the Kinkel et al. (1965) study extensive neurohistological examinations did not reveal treatment-related alterations (C.H. Boehringer Sohn, 1968). In a 100-day study, groups of 1, 2, 2, and 2 dogs of each sex received capsules that provided 0, 0.75, 1.5 and 3.0 mg bromophos/kg bw/day (Muacevic et al., 1970; Kinkel and Dirks, 1966). Equivocal slight or no decreases in plasma or erythrocyte levels occurred throughout the study and 7 weeks postexposure, but liver and brain cholinesterase were normal when assayed 1 and 7 weeks postexposure.

6. AQUATIC TOXICITY

6.1. ACUTE

Alabaster (1969) reported 24- and 48-hour LC_{50} values for the harlequin fish, Rasbora heteromorpha, to be 1.2 and 0.62 mg/l, respectively, in a static bioassay. Worthing and Walker (1983) reported that the 24-hour LC_{50} for the guppy, Poecilia reticulata, was 0.55 mg/l. Concentrations of 0.5-1.0 mg/l bromophos were not lethal to the mosquitofish, Gambusia affinis, in its natural surroundings (Worthing and Walker, 1983). A concentration of 1 ppm was not lethal to Bufo bufo tadpoles exposed for 72 hours (Pimental, 1971).

The MAD for reducing the number of generations based on inhibition of growth of the ciliate, Colpidium campylum, in monoxenic culture was 10 and 5.6 mg/l for bromophos-ethyl and -methyl, respectively (Dive et al., 1980). Kneffel et al. (1975, 1976) proposed using changes in motility of larvae (glochidia) of the lacustrine mussel, Anodonta cygnea, as a sensitive assay for aquatic toxicity. Bromophos caused measurable changes in frequency of contractions of constrictor muscles at concentrations of 3-10 mg/l (Kneffel et al., 1975). The threshold NOEC for this effect is 0.1 μ g/l (Kneffel et al., 1976), but the relevance of this effect to other toxic effects is not readily apparent.

Siekel et al. (1979) reported a 24- to 48-hour LC_{50} of 0.2 mg/l for the mosquito larvae, Culex pipiens molestus, while Rettich (1979) reported an LC_{100} of 0.01 mg/l for mosquito larvae of the same species. Rettich (1979) also reported 24- and 48-hour LC_{50}/LC_{90} values of 0.010/0.047 mg/l and 0.006/0.025 mg/l, respectively. An LC_{50} value of 0.0064 mg/l was reported for Daphnia pulex (Dive et al., 1980).

Oteifa et al. (1975) investigated the toxicity of various insecticides, including Nexone (bromophos), to the freshwater snails, Blomphalaria alexandrina and Bulinus truncatus. The 24-hour LC_{50} and LC_{90} values for juveniles and adults range from 2.64-10.0 mg/l and are shown in Table 6-1. LC_{100} values for egg masses of the two species were 10.0 mg/l for B. alexandrina and 8.0 mg/l for B. truncatus.

6.2. CHRONIC

Pertinent data regarding the chronic toxicity of bromophos to aquatic organisms could not be located in the available literature as cited in the Appendix.

6.3. PLANTS

Bromophos at an unspecified concentration inhibited growth and photosynthesis in the alga, Chlorella. Chlorophyll was synthesized in this alga at a bromophos concentration of 0.005 mg/l (Petrya and Spirescu, 1977). Data regarding rooted plants could not be located in the available literature as cited in the Appendix.

6.4. RESIDUES

Pertinent data regarding residues of bromophos in aquatic organisms could not be located in the available literature as cited in the Appendix.

6.5. SUMMARY

Little information is available concerning the toxicity of bromophos to aquatic organisms. The acute LC_{50} value for fish is ~0.5 mg/l. Based on limited data, bromophos appears to be 1-2 orders of magnitude more acutely toxic to aquatic invertebrates and to Chlorella than to fish.

TABLE 6-1

24-Hour LC₅₀ and LC₉₀ Values for the Freshwater Snails,
Biomphalaria alexandrina and Bulinus truncatus*

Species	Life Stage	LC ₅₀ (mg/l)	LC ₉₀ (mg/l)
<u>Biomphalaria alexandrina</u>	newly hatched	5.6	8.6
	adult	5.8	10.0
<u>Bulinus truncatus</u>	newly hatched	7.3	9.8
	adult	2.64	5.08

*Source: Otelfa et al., 1975

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

The WHO/FAO has established a temporary ADI for man of 0.006 mg bromophos/kg bw (WHO/FAO, 1973). Establishment of a permanent ADI was precluded by poor quality of available data. This ADI appears to be based on the 100-day oral study with rats that showed no effects on plasma cholinesterase at 0.65 mg/kg/day (Leuschner and Leuschner, 1966a).

7.2. AQUATIC

Guidelines and standards for the protection of aquatic organisms from the toxic effects of bromophos could not be located in the available literature as cited in the Appendix.

8. RISK ASSESSMENT

Bioassays designed specifically for the assessment of carcinogenicity of bromophos have not been conducted. Studies in which bromophos was administered by gavage to groups of 25 male and 25 female rats at doses of 0, 87.5, 175 or 350 mg/kg or to groups of 3 male and 3 female dogs at doses of 0, 11, 43.8, 87.5 and 175 mg/kg each workday for up to 2 years (Kinkel et al., 1965) provided no indication of neoplasm development. It should be noted, however, that these studies are unpublished and were available only as summaries in a secondary source (WHO/FAO, 1973); although gross and histological examinations were reported to have been conducted, the extent of the pathological examinations was not specified. It also should be noted that the rats were 6 months old at the start of the study and that because of poor survival an additional 3, 5, 5 and 17 rats were added in the 0, 87.5, 175 and 350 mg/kg treatment groups, respectively, at an unspecified time after the start of the study, and that small numbers of dogs were tested.

Bromophos was nonmutagenic in reverse mutation assays with five strains of *S. typhimurium* (Lippens et al., 1983), in a forward mutation assay with the yeast, *S. pombe* strain SP-198 (Gilot-Delhalle et al., 1983; Degraeve et al., 1980), in a recessive lethal mutation assay with *D. melanogaster* (Benes and Sram, 1969) and in a dominant lethal mutation assay with intraperitoneally injected mice (Degraeve et al., 1980, 1984a). Intraperitoneal injection of bromophos into mice also produced no chromosomal aberrations in the bone marrow cells or spermatogonia (Degraeve et al., 1984a,b).

Oral (gavage) administration of bromophos to rabbits on days 6-18 of gestation at dosages of 25, 50, 100, 200 or 400 mg/kg/day did not produce an increase in the type or incidence of fetal malformations (Leuschner and

Leuschner, 1966a). Effects indicative of maternal toxicity (debilitation and limb flaccidity) and fetotoxicity (reduced number of full grown live fetuses) occurred, however, at ≥ 50 mg/kg/day. Administration of diets providing 5, 20 or 80 mg/kg/day to rats during a 3-generation study did not produce malformations in any of the offspring (Leuschner et al., 1967). Although these results provide evidence that bromophos is not teratogenic, it should be noted that the studies are unpublished and that endpoints and results were reported inadequately in a secondary source.

Since data regarding the carcinogenicity of bromophos are inadequate, it is appropriate to use non-neoplastic endpoints to derive an ADI. Examination of the chronic and subchronic toxicity, teratogenicity and other reproductive effects data summarized in Sections 5.3., 5.4. and 5.5. indicates that cholinesterase inhibition can be used to identify NOAELs and a LOAEL for bromophos. All of the relevant studies of bromophos assayed plasma and erythrocyte cholinesterase activities, most assayed brain cholinesterase and many also assayed liver cholinesterase. It is necessary to recognize, however, that the only cholinesterase inhibition with any known functional significance is that within neuroeffector junctions or the effector itself (Wills, 1972). Significant inhibition of plasma or erythrocyte cholinesterase is regarded as suggestive of exposure to a cholinesterase inhibitor, but not necessarily indicative of interference with normal function of neuroeffector systems. Hence, doses associated with plasma, erythrocyte or liver cholinesterase inhibition most appropriately represent NOAELs, and doses associated with inhibition of brain cholinesterase should be regarded as LOAELS.

It should be noted that the preponderance of chronic and subchronic toxicity studies of bromophos are unpublished and available only as summaries in a WHO evaluation (WHO/FAO, 1973). Treatment information was usually

adequately reported, but deficiencies regarding other aspects of experimental design (i.e., indices examined) as well as results (i.e., lack of quantitation) were frequently apparent [with the exception of the Muacevic et al. (1970) report]. The information used for ADI derivation, therefore, reflects the deficiencies of the available data.

In chronic studies of bromophos in rats and subchronic studies in dogs, groups of 25 male and 25 female rats were exposed by gavage to doses of 0, 87.5, 175 or 350 mg/kg and groups of 3 male and 3 female dogs were exposed by gavage to doses of 0, 11, 43.8, 87.5 and 175 mg/kg on each workday (5 days/week) for up to 2 years (Kinkel et al., 1965). Because of poor survival, an additional 3, 5, 5 and 17 rats were included in the 0, 87.5, 175 and 350 mg/kg groups, respectively, at an unspecified time after the start of the experiment. Expanding the exposures over a 7-day week results in equivalent daily doses of 0, 62.5, 125 and 250 mg/kg/day in the rat study and 0, 7.9, 31.3, 62.5 and 125 mg/kg/day in the dog study. Plasma, erythrocyte, liver and brain cholinesterases were reported to be inhibited at all dose levels in both the rats and the dogs; other effects (reduced body weight, proteinuria and hematuria in the rats, signs of intoxication with some deaths and impaired spermatogenesis in the dogs) occurred only at the highest doses. The results of other experiments (Muacevic et al., 1970) by the same group of investigators with identical dosages and treatment, apparently differing only in numbers of animals (10 or 11 rats of each sex/dose, 5 or 6 dogs of either sex/dose, except 1 dog of unspecified sex in the high-dose group), method of oral administration (capsule in diet) and treatment schedule (daily exposure) in the dog study, are consistent with these findings. In the rats, plasma, liver and brain cholinesterase were strongly inhibited (82-84%, 62-79% and 73-79% of control values, respectively) at all doses, but erythrocyte cholinesterase was not strongly

inhibited at any dose (23-47% of control values). In the dogs, brain and liver cholinesterase were inhibited at all doses (57-65% and 83-97% inhibition, respectively), and erythrocyte and plasma cholinesterase were inhibited (98-99% and 70-81%, respectively) at all but the lowest dose (32 and 27%, respectively).

Diets providing 50 mg bromophos/kg bw/day for 42 days produced brain cholinesterase inhibition in rats, but this effect was not statistically significant at 1, 3 or 10 mg/kg/day (Desl et al., 1971); significant plasma cholinesterase inhibition was evident at 50 mg/kg/day, and erythrocyte cholinesterase inhibition occurred at 10 and 50 mg/kg/day. Related effects in this study included dose-related decrements in performance in a conditioned reflex test from the lowest dose, and lowered EEG activity at 50 mg/kg/day; the other effects at 50 mg/kg/day included significantly increased liver, kidney and dried cerebral weights and indications of hepatic granular cell degeneration and epithelial tubular cell swelling in the kidneys. Brain cholinesterase levels were not determined in a 100-day dietary study with rats that ingested 0.65, 1.25 or 2.5 mg/bromophos/kg bw/day (Leuschner and Leuschner, 1966b); erythrocyte cholinesterase was not reduced in either sex, and plasma cholinesterase was reduced only in female rats exposed to 2.5 mg/kg/day. Dogs exposed to diets providing 0, 0.75, 1.5 or 3.0 mg bromophos/kg bw/day for 100 days had equivocal decreases or no decreases in erythrocyte and plasma cholinesterases during exposure and the 7-week post-exposure period (Muacevic et al., 1970; Kinkel and Dirks, 1966). Brain and liver cholinesterase were reported to be normal, but assays were conducted only 1 and 7 weeks postexposure. Although these data appear to indicate a lack of brain cholinesterase inhibition at all dosages, the doses should be regarded as equivocal NOAELs because the lack of postexposure brain cholinesterase reduction does not preclude the possibility of reduced brain

cholinesterase during treatment and because very small numbers of dogs were assayed (one or two dogs of each sex/dose).

The only study which both monitored for the critical adverse effect (brain cholinesterase inhibition) and defined a NOAEL is the 3-generation reproduction study (Leuschner et al., 1967). In this study parental animals were examined after 6 months of exposure to dietary bromophos and F₁ and F₂ animals after 8 months. For all three generations, 5 mg/kg/day (authors estimate) appeared to be a NOAEL with depression of plasma and liver cholinesterase but not brain or erythrocyte cholinesterase. The 20 mg/kg/day dose appeared to represent a LOAEL for males and a NOAEL for females. Erythrocyte cholinesterase was depressed and brain cholinesterase was depressed in males. At 80 mg/kg/day brain cholinesterase was depressed in females.

Although interpretation of the available studies is hindered as a result of the necessity of utilizing secondary sources, the data are relatively consistent. The NOAEL from the multigeneration study of 5 mg/kg bw is supported by a NOAEL of 10 mg/kg/day in a 42-day rat study (Desi et al., 1971). The data of Muacevic et al. (1970) and Kinkel and Dirks (1966) suggest a NOAEL for brain cholinesterase inhibition in dogs at 3 mg/kg/day. The flaws in this study have been previously discussed. In addition, effects other than cholinesterase inhibition have not been reported except at substantially higher doses.

An ADI can be developed based on the NOAEL of 5 mg/kg/day. Dividing by an uncertainty factor of 1000 (10 for using a subchronic study, 10 for interspecies extrapolation and 10 for interindividual variability) results in an estimated ADI of 0.005 mg/kg/day or 0.35 mg/day for a 70 kg man. This value compares favorably with the temporary ADI estimate by WHO/FAO (1973) of 0.006 mg/kg.

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

The available chronic, subchronic, teratogenicity and reproductive studies of bromophos were discussed in Chapter 5 and are summarized in Table 9-1. The preponderance of chronic and subchronic toxicity studies of bromophos, however, are unpublished and available only as summaries in a WHO evaluation (WHO/FAO, 1973). Treatment information was usually adequately reported, but deficiencies regarding other aspects of experimental design (i.e., indices examined) as well as results (i.e., lack of quantitation) were frequently apparent [with the exception of the Muacevic et al. (1970) report]. The information used for RQ derivation, therefore, reflects the deficiencies of the available data.

Administration of bromophos to rats by gavage at a transformed dose of 250 mg/kg/day for 2 years caused proteinuria and hematuria (Kinkel et al., 1965); lower doses (62.5 and 125 mg/kg/day) did not cause these effects, and histological or organ weight alterations were not observed at any dose. In subchronic studies of rats exposed to bromophos doses of 188, 750 or 1250 mg/kg/day, reduced body weight gain occurred at all doses with lowered food consumption only at the high dose, while dose-related hydropic swelling of the liver cells at all doses and proteinuria at the high dose also occurred (Kinkel and Seume, 1963). Increased liver, kidney and dried cerebral weights occurred in rats exposed to diets that provided 50 mg bromophos/kg bw/day, but body weight was normal and these effects were not seen at 1, 3 or 10 mg/kg/day (Desi et al., 1971). Granular cell degeneration in the liver and tubule epithelial cell swelling in the kidney were noted, but incidences were not significant for the liver and not reported for the kidney. These effects in rats at relatively low doses for 42 days were not

TABLE 9-1
Toxicity Summary for Bromophos^a

Route	Species/ Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Exposure	Transformed Animal Dose (mg/kg/day)	Response	Reference
Oral (gavage)	rat/NR	M,F	25/sex/dose ^b	NR	NR	87.5, 175 or 350 mg/kg, 5 days/week for 2 years	62.5, 125 or 250	Reduced body weight gain proteinuria and hema- turia at 250 mg/kg/day. Reduced plasma, erythro- cyte, brain and liver cholinesterase at all dosages. Unspecified hematological and liver function indices, ascorbic acid excretion and histology of kidneys and other unspecified organs unremarkable. Weight of eight unspec- ified organs normal.	Kinkel et al., 1965
Oral (gavage)	rat/FM-49	M,F	10 or 11/ sex/dose	NR	3% Mulsifar [®] (emulsifier)	87.5, 175 or 350 mg/kg, 5 days/week for 2 years	62.5, 125 or 250	Reduced plasma, liver and brain cholinesterase activities. Erythrocyte cholinesterase not strongly inhibited. Responses not clearly dose-related and com- parable among groups.	Muacevic et al., 1970
Oral (gavage)	rat/NR	M	16/dose	NR	NR	188, 750 or 1250 mg/kg/day for 100 days	188, 750 or 1250	Reduced body weight gain at all doses with lowered food consumption only at high dose. Re- duced plasma, liver and brain cholinesterase and hydropic swelling of liver cells at all doses, but only hydropic swell- ing dose-related. Hy- aline droplets in renal tubular cells and protein in the tubules at high dose. Unspecified urinalysis and hemato- logical indices, bromo- sulphthalein retention and gross appearance of unspecified organs normal.	Kinkel and Seume, 1963

TABLE 9-1 (cont.)

Route	Species/ Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Exposure	Transformed Animal Dose (mg/kg/day)	Response	Reference
Oral	rat/Mistar	M,f	6/sex/dose	NR	diet	0.65, 1.25 or 2.5 mg/kg bw/day for 100 days	0.65, 1.25 or 2.5	Reduced plasma choline- terase activity in high- dose females, but no effect in males or on erythrocyte choline- terase in either sex. Normal behavior, growth, food intake and gross appearance of unspec- ified organs.	Leuschner and Leuschner, 1966a
Oral	rat/NR	F	8/dose	0.10-0.12	diet	0, 10, 30, 100 and 500 ppm for 42 days	0, 1, 3, 10 and 50 (reported)	Significantly reduced erythrocyte choline- terase at 10 and 50 mg/kg/day and plasma and brain choline- terase at 50 mg/kg/day. Significantly increased liver, kidney and dried cerebral weights at 50 mg/kg/day, but no effect on body weight. Liver granular cell degenera- tion and kidney epithe- lial tubule cell swell- ing suggested, but in- cidence not significant (liver) or not reported (kidney). No histologic alteration in the brain. No treatment-related effect on performances in a labyrinth or orien- tation reaction tests (numbers tested in latter not specified).	Desl et al., 1971

TABLE 9-1 (cont.)

Route	Species/ Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Exposure	Transformed Animal Dose (mg/kg/day)	Response	Reference
Oral	rat/NR	F	8/dose	0.10-0.12	diet	0, 10, 30, 100 and 500 ppm for 42 days	0, 1, 3, 10 and 50 (reported)	Different groups of rats from above. Dose- related decrement in performance in a condi- tioned reflex test. No effect on body weight.	Desl et al., 1971
Oral	rat/NR	F	16 in each treated group, 20 controls	0.01-0.12	diet	0, 100 and 500 ppm for 42 days	0, 10 and 50 (reported)	Different groups of rats than above. Significant reduction in brain ac- tivity as indicated by EEG at 50 mg/kg/day. Results of histological examination pooled with the cholinesterase study detailed above.	Desl et al., 1971
Oral	rat/NR	M, F	20/sex/dose	NR	diet	0, 5, 20 and 80 mg/kg bw/day (3-generation reproduction study)	0, 5, 20 and 80	Decreased parental body weight gain, increased incidence of stillbirths and reduced neonatal sur- vival at 80 mg/kg/day. Plasma and liver cholin- esterase inhibited at 5 mg/kg/day, erythrocyte cholinesterase inhibited at 20 mg/kg/day, and brain cholinesterase in- hibited at 20 mg/kg/day (males) and 80 mg/kg/day (females) in parental and filial rats when assayed after 6 and 8 months of exposure, respectively.	Leuschner et al., 1967

TABLE 9-1 (cont.)

Route	Species/ Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Exposure	Transformed Animal Dose (mg/kg/day)	Response	Reference
Oral (gavage)	dog/mongrel	M, F	3/sex/dose	3.9-10.3	MR	11, 43.8, 87.5 and 175 mg/kg, 5 days/week for 2 years	7.9, 31.3, 62.5 or 125	Respiratory difficulty, salivation, tremors, ataxia and hindlimb paresis in 5/6 high- dose animals; 3/6 died (sex not indicated). Similar effects and death in 1/6 dogs in 62.5 mg/kg/day group. Spinal cord ganglion degeneration in dogs that died, but other histologic alterations not indicated. Impaired spermatogenesis in 2/3 high-dose males but "sexual cycle" normal in females. Plasma, eryth- rocyte, liver and brain cholinesterase activi- ties depressed at all dose levels. No effects on growth, food intake, urinary ascorbic acid ex- cretion, or unspecified hematologic, liver func- tion, and other urinary indices.	Kinkel et al., 1965
Oral	dog/mongrel	M, F	6, 6, 5, 5 and 1 dogs of either sex in control to high-dose groups, re- spectively	3.9-10.3	diet (capsule)/ suspension with 3% Mulfafarine	0, 11, 43.8, 87.5 and 175 mg/kg/day for 2 years	0, 11, 43.8, 87.5 and 175	Cholinesterase inhibi- tion in erythrocyte and plasma at all but the lowest dose and in brain and liver at all doses. Inhibition marked but not dose-related.	Muscevic et al., 1970

TABLE 2-1 (cont.)

Route	Species/ Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Exposure	Transformed Animal Dose (mg/kg/day)	Response	Reference
Oral	dog/mongrel	M, F	3, 3 and 6 dogs of each sex in control to high-dose groups, re- spectively	6-20	diet (capsule)/ NR	0, 0.75 and 175 mg/kg bw/day for 270 days	0, 0.75 and 175	Dose-related reductions in body weight and food consumption. Reduced plasma and erythrocyte cholinesterase in both groups. Seminiferous tubule degeneration in 3/6 males at 175 mg/kg/ day and unquantified evi- dence of prostatic hyper- trophy in all groups. Unspecified hematologic serum and urinalyses normal. Neurohistology unremarkable.	C.H. Boehringer Sohn, 1968
Oral	dog/beagle	M, F	1, 2, 2 and 2 dogs of each sex in control to high-dose groups, re- spectively	7.7-12.6 (initial)	diet (capsule)/ NR	0, 0.75, 1.5 and 3.0 mg/kg bw/day for 100 days	0, 0.75, 1.5 and 3.0	Equivocal or no decrease in erythrocyte and plasma cholinesterase throughout test (all dogs tested) and during 7-week post- exposure period (half the dogs tested). Brain and liver cholinesterase reportedly normal at 1 (half the dogs) and 7 (remaining dogs) weeks postexposure, but data not provided.	Muacevic et al., 1970; Kinkel and Birks, 1966
Oral (gavage)	rabbit/NR	F	10 in each treated group, 40 controls	NR	NR	0, 25, 50, 100, 200 and 400 mg/kg/day on days 6-18 of gestation	0, 25, 50, 200 and 400	Maternal toxicity (debilitation, limb flaccidity, diarrhea) and fetotoxicity (re- duced number of fully grown live fetuses) at doses >50 mg/kg/day. No evidence of teratogenicity.	Leuschner and Leuschner, 1966a

aPurity not reported

b3, 5, 5 and 17 rats were included in the 0, 0.75, 175 or 350 mg/kg groups, respectively, after the start of the experiment, but additional information (i.e., time of addition) was not reported.

cSex distribution in treatment groups not specified.

NR = Not reported

supported by longer-term studies using higher doses; they will not be considered for RQ derivation. It does not appear to be appropriate to calculate a CS for the effects of hydropic swelling of liver cells at ≥ 188 mg/kg/day for 100 days in the study of Kinkel and Seume (1963), since this effect apparently was not observed at 250 mg/kg/day for 2 years in a study from the same laboratory (Kinkel et al., 1965). Hyaline deposits in renal tubule cells were observed at 1250 mg/kg/day in the 100-day study, but this effect is no more severe than hydropic swelling. It is more appropriate to calculate a CS based on the effects of hematuria and proteinuria at 250 mg/kg/day in the Kinkel et al. (1965) study; proteinuria occurred at 750 mg/kg/day in the 100-day study.

Another effect of bromophos treatment was inhibition of cholinesterase. Since the only cholinesterase inhibition with functional significance is that within neuroeffector junctions or the effector itself (Wills, 1972), inhibition of brain cholinesterase is a more serious effect than inhibition of erythrocyte, plasma or liver cholinesterase. The lowest doses at which brain cholinesterase occurred were 20 mg/kg/day in the 3-generation rat study by Leuschner et al. (1967) and 7.9 mg/kg/day in the 2-year dog study by Kinkel et al. (1965); erythrocyte, plasma and liver cholinesterase activities were also inhibited at ≥ 7.9 mg/kg/day in dogs. The lowest rat dose for inhibition of plasma cholinesterase is 2.5 mg/kg/day in the 100-day study by Leuschner and Leuschner (1966b). These effects will be considered for RQ derivation. The EEG alterations and conditioned reflex decrements in the Desi et al. (1971) study were not considered valid endpoints for RQ calculation because the toxicological significance of these effects is uncertain and a lack of corroborating data for behavioral/neurophysiological effects from other studies.

Dogs exposed to 125 mg bromophos/kg/day experienced signs of acute intoxication (e.g., tremors, ataxia, paresis), which resulted in early deaths of 3/6 dogs, histological evidence of neurological damage (spinal cord ganglion degeneration) in the dogs that died and impaired spermatogenesis in 2/3 males (Kinkel et al., 1965). Similar signs of intoxication and death occurred in 1/6 dogs exposed to the next lower dose (62.5 mg/kg/day); these effects appear to be acute responses to relatively higher doses, indicating unsuitability for MED calculation. In another study, 3/6 male dogs exposed to diets that provided bromophos at doses of 175 mg/kg/day for 270 days had seminiferous tubule degeneration (C.H. Boehringer Sohn, 1968). Prostatic hypertrophy occurred in these dogs as well as in dogs exposed to 87.5 mg/kg/day, but the incidences of this effect were not reported. It does not appear to be appropriate to calculate a MED from the 87.5 mg/kg/day dose since more serious effects were evident in dogs at a slightly lower dose (62.5 mg/kg/day) in the Kinkel et al. (1965) study.

Bromophos treatment also resulted in reduced body weight gain in rats at doses ≥ 80 mg/kg/day and in dogs at ≥ 87.5 mg/kg/day (see Table 9-1). Since more severe effects occurred at lower doses, however, it is not necessary to calculate CSs for reduced body weight gain.

Diets that provided 80 mg bromophos/kg bw/day to rats in the Leuschner et al. (1967) 3-generation study were toxic to the fetuses (unspecified high incidence of stillbirths) and neonates (increased mortality), but not teratogenic. Maternal toxicity (debilitation, limb flaccidity, diarrhea) and fetotoxicity (reduced number of full grown live fetuses) without teratogenicity was observed in rabbits exposed to ≥ 50 mg/kg/day bromophos by gavage on days 6-18 of gestation (Leuschner and Leuschner, 1966a). Human MEDs can be calculated from these endpoints in both of these species.

The data used to calculate CSs are given in Table 9-2. The MEDs were calculated from the transformed animal doses by multiplying by the cube root of the ratio of the animal weight to the reference weight of a human (assumed to be 70 kg). The weights used were 0.35 kg for rats (U.S. EPA, 1980), 3.8 kg for rabbits (Durkin, 1985) and 7.1 kg for the 1- to 3-year-old mongrel dogs, the midpoint of the starting weight range reported by Kinkel et al. (1965). Doses were divided by 10 when based on subchronic animal data. The resulting human doses in mg/kg/day were converted to MEDs in units of mg/day by multiplying by 70 kg. The RV_d s corresponding to the MEDs and the most appropriate RV_e s are given in Table 9-2. The highest CS was 20.6, resulting from the data for brain cholinesterase inhibition in rats (Leuschner et al., 1967) (Table 9-3). This CS corresponds to an RQ of 100 (see Table 9-2). It should be noted that the deficiencies of the data base for bromophos (i.e., preponderance of secondary summaries of unpublished studies with minimal data, small group sizes in the dog studies) are mitigated by the corroboration of the rat and dog CSs based on brain cholinesterase inhibition.

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

Studies designed specifically for the evaluation of the carcinogenicity of bromophos have not been conducted. Gross and histological examinations of rats exposed to doses of 87.5, 175 and 350 mg/kg or dogs exposed to doses of 11, 43.8, 87.5 and 175 mg/kg by gavage, each workday for 2 years did not indicate tumor development (Kinkel et al., 1965). It should be noted that the rats were 6 months old at the start of the experiment with consequent poor survival and that small numbers of dogs (three/sex/dose) were tested.

TABLE 9-2
Composite Scores for Bromophos

Route	Species	Animal Dose (mg/kg/day)/ Exposure	Chronic Human MED (mg/day)	RV _d	Effect	RV _e	CS	RQ	Reference
Oral (gavage)	rat	250/2 years	2993	1.0	proteinuria and hematuria	5	5.0	5000	Kinkel et al., 1965
Oral (diet)	rat	20/6-8 months	25.9*	3.43	brain cholinesterase inhibition	6	20.6	100	Leuschner et al., 1967
Oral	rat	2.5/100 days	3*	4.8	inhibition of plasma, erythro- cyte or liver cholinesterase	2	9.6	1000	Leuschner and Leuschner, 1966b
Oral (diet)	rat	80/gestation	958	1.0	stillbirths and increased neonatal mortality	10	10	1000	Leuschner et al., 1967
Oral (gavage)	rabbit	50/gestation days 6-18	1325	1.0	fetotoxicity (reduced number of full grown live fetuses) and maternal toxicity	8	8.0	1000	Leuschner and Leuschner, 1966a
Oral (gavage)	dog	7.9/2 years	25.8*	3.38	brain cholinesterase inhibition	6	20.3	1000	Kinkel et al., 1965

*factor of 10 applied to derive a chronic MED based on subchronic data.

TABLE 9-3

Bromophos

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose*:	23.9 mg/kg/day
Effect:	brain cholinesterase inhibition
Reference:	Leuschner et al., 1967
RV _d :	3.43
RV _e :	6
Composite Score:	20.6
RQ:	100

*Equivalent human dose

Bromophos was not mutagenic in reverse mutation assays with five strains of S. typhimurium (Lippens et al., 1983), in a forward mutation assay with the yeast, S. pombe, strain SP-198 (Gilot-Delhalle et al., 1983; Degraeve et al., 1980), in a recessive lethal mutation assay with D. melanogaster (Benes and Sram, 1969) and in a dominant lethal mutation assay with intraperitoneally injected mice (Degraeve et al., 1980, 1984a). Intraperitoneal injection of bromophos into mice also did not produce chromosomal aberrations in the bone marrow cells or spermatogonia (Degraeve et al., 1984a,b).

Using the IARC criteria for the weight of evidence of carcinogenicity, bromophos is classified as a Group 3 chemical (negative experimental evidence). Under the guidelines proposed by U.S. EPA Cancer Assessment Group, bromophos is classified as a Group D chemical (inadequate evidence of carcinogenicity) since no bioassays designed for the assessment of carcinogenicity of bromophos have been conducted.

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APPENDIX
LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

CA SEARCH (Files 308, 309, 310, 311, 320)
TOXLINE
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP

These searches were conducted in August, 1985 with the exception of the TOXLINE, which was searched in February, 1984 and was kindly supplied by Dynamac Corp. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1980. Documentation of the Threshold Limit Values, 4th ed. (Includes Supplemental Documentation, 1981, 1982, 1983). Cincinnati, OH. 486 p.

ACGIH (American Conference of Governmental Industrial Hygienists). 1985. TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1985-1986. Cincinnati, OH. 114 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2A. John Wiley and Sons, NY. p. 2878.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2B. John Wiley and Sons, NY. p. 2879-3816.

Clayton, G.D. and F.E. Clayton, Ed. 1982. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2C. John Wiley and Sons, NY. p. 3817-5112.

Grayson, M. and D. Eckroth, Ed. 1978-1983. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. John Wiley and Sons, NY. 23 Volumes.

Hamilton, A. and H.L. Hardy. 1974. Industrial Toxicology, 3rd ed. Publishing Sciences Group, Inc., MA. 575 p.

IARC (International Agency for Research on Cancer). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. WHO, IARC, Lyons, France.

ITII (International Technical Information Institute). 1982. Toxic and Hazardous Industrial Chemicals Safety Manual for Handling and Disposal with Toxicity and Hazard Data. ITII, Tokyo, Japan. 700 p.

NTP (National Toxicology Program). 1984. Toxicology Research and Testing Program. Chemicals on Standard Protocol. Management Status.

Ouellette, R.P. and J.A. King. 1977. Chemical Week Pesticide Register. McGraw-Hill Book Co., NY.

Sax, N.I. 1979. Dangerous Properties of Industrial Materials, 5th ed. Van Nostrand Reinhold Co., NY.

SRI (Stanford Research Institute). 1984. Directory of Chemical Producers. Menlo Park, CA.

U.S. EPA. 1985. Status Report on Rebuttable Presumption Against Registration (RPAR) or Special Review Process. Registration Standards and the Data Call in Programs. Office of Pesticide Programs, Washington, DC.

U.S. EPA. 1985. CSB Existing Chemical Assessment Tracking System. Name and CAS Number Ordered Indexes. Office of Toxic Substances, Washington, DC.

USITC (U.S. International Trade Commission). 1983. Synthetic Organic Chemicals. U.S. Production and Sales, 1982, USITC Publ. 1422. Washington, DC.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY.

Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

Windholz, M., Ed. 1983. The Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

Battelle's Columbus Laboratories. 1971. Water Quality Criteria Data Book. Volume 3. Effects of Chemicals on Aquatic Life. Selected Data from the Literature through 1968. Prepared for the U.S. EPA under Contract No. 68-01-0007. Washington, DC.

Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. USDI, Fish and Wildlife Serv. Res. Publ. 137, Washington, DC.

McKee, J.E. and H.W. Wolf. 1963. Water Quality Criteria, 2nd ed. Prepared for the Resources Agency of California, State Water Quality Control Board. Publ. No. 3-A.

Pimental, D. 1971. Ecological Effects of Pesticides on Non-Target Species. Prepared for the U.S. EPA, Washington, DC. PB-269605.

Schneider, B.A. 1979. Toxicology Handbook. Mammalian and Aquatic Data. Book 1: Toxicology Data. Office of Pesticide Programs, U.S. EPA, Washington, DC. EPA 540/9-79-003. NTIS PB 80-196876.